

A PRELIMINARY STUDY ON ASSESSING BODY BURDEN OF PERSISTENT ORGAINIC POLLUTANTS (POPs) IN INFANTS THROUGH ANALYSIS OF FAECES

Chen YQ¹, Li Y¹, Toms LML², Gallen M¹, Hearn L¹, Sly PD³, Mueller JF¹

¹ National Research Centre for Environmental Toxicology, The University of Queensland, Australia; ² School of Clinical Sciences and Institute of Health and Biomedical Innovation, Faculty of Health, Queensland University of Technology, QUT, Australia; ³ Queensland Children's Medical Research Institute, University of Queensland, Australia

Introduction

Most persistent organic pollutants (POPs) like polychlorinated biphenyls (PCBs), a range of polybrominated diphenyl ethers (PBDEs) and organochlorine pesticides (OCPs) are readily absorbed (via the ingestion and inhalation) and accumulate in fatty tissue, including adipose tissue and human milk [1]. Health effects related to exposure to these chemicals may include neurological effects, altered functioning of the nervous system and/or endocrine disruption [2-4]. The burden of environmental disease is recognized as much higher for children than adults, especially in young children under 5 years of age worldwide [5]. There is increased concern regarding the environmental impact on the health of children who have been disproportionately affected by environmental problems. For example they may be subjected to relatively higher exposure, have greater physiological susceptibility and/or suffer more extreme consequences due to growth [6-9]. It is therefore worthwhile to assess the correlation between burden of disease and exposure to xenobiotic chemical pollutants like POPs. Such assessment may provide guidance for legislative changes regarding chemical bans and give reliable advice to parents including lactating mothers.

The conventional technique of measuring body burden for POPs is via collection and analysis of serum. However with children, collection of sufficient serum for analysis of trace organic pollutants remains typically 'too difficult' with respect to ethics, consent/participation and feasibility (i.e. requirement for multiple sampling to get sufficient volume) [10]. This problem with sample volume has been overcome in some studies by pooling samples from groups of individuals, however, this approach is not suitable for longitudinal assessments of body burden during infancy and early childhood when exposure is least understood.

For a range of persistent lipophilic organic pollutants, it has been shown that desorption from the body into the lumen of the digestive tract followed by fecal excretion is one of the major mechanisms for physical elimination [11-12]. Early evidence came from studies on breast fed children which showed that faecal excretion of polychlorinated dibenzodioxins and furans (PCDD/PCDFs) did not change/decrease over the weaning period despite the fact that exposure/uptake via breast milk was estimated to be much higher than in the substituted diet [13]. Moser and McLachlan further suggested that the fecal levels of a range of POPs are determined by their concentration in the body, not in the diet [16]. A feces/blood distribution coefficient K_{FB} was defined as the quotient of the dry weight-based concentration in feces and the lipid-based concentration in blood demonstrating that the dry weight-based faeces concentration can be linearly related to the lipid-based blood concentration over a very wide range. Therefore, with the use of K_{FB} , fecal POPs have the potential to make a reliable biomarker for the body burden of POPs. McLachlan and team undertook a range of systematic studies expanding the concept with the aim to increase clearance of POPs using non-digestible fat, however the focus of their work was primarily on parameterizing a model for the uptake and clearance of POPs in humans and not on applying the approach to estimating body burden.

With the present study we aim to quantify POPs in faeces of infants/toddlers. Furthermore, in this preliminary study we aim to compare the measured concentrations of POPs in the faeces to recent Australian data from pooled children (0-4 years in age) blood serum [15].

Materials and methods

Sample Collection and Storage

For this study, we recruited 5 infants and 2 toddlers from families of at least two years' residence in Brisbane, Australia, who originate from several countries (Table 1).

Table 1 Demographics of infants/toddlers including national background of parents, year of birth, age of mother and child at sample collection and feeding status

Subject	Gender	Parents' nationality/Residence years in Australia	The year of birth	Mother's age at the year of birth	Primipara or other	Age at the time of sampling	Feeding component at the time of sampling
Infant 1	Male	Canada/2 years	2012	34	Primipara	6 month-11 months	Breast milk/formula milk/solid food
Infant 2	Male	New Zealand/3 years	2012	33	Primipara	5-6 months	Breast milk/solid food
Infant3*	Female	Malaysia & Chinese/4 years	2012	36	Second	5-6 months	Breast milk/formula milk
Infant 4	Female	Australia	2012	32	Primipara	7 months	Breast milk/formula milk/solid food
Infant 5	Female	Australia	2012	40	Primipara	7months	Breast milk/formula milk/solid food
Toddler 1	Female	Australia	2010	33	Primipara	2 years	Solid food
Toddler2*	Male	Malaysia & Chinese/4 years	2010	34	Primipara	2 years and half	Formula milk/solid food

*Toddler 2 and Infant 3 are from a vegetarian family.

Parents collected faecal samples twice a week for one month for each individual child. Sample collections were done by directly taking a portion of the faeces that was not in contact with the nappy (or from a liner applied on top of the nappy which can help retain a dry portion of the faeces). Nappy and liner materials were also analyzed as QCQA samples with very low concentration of POPS observed. Samples were freeze-dried and stored at -80 °C in clean amber jars covered by PTFE-lined screw lid until extraction.

Sample extraction and clean-up

3 gram aliquots of the dried faeces samples as well as a blank consisting of bovine calf serum were placed in a diodonium cell with 3 grams of hydromatirx. The samples were then spiked with internal standards and extracted using n-hexane in an Accelerated Solvent Extractor (ASE), at 100 °C, 1500 psi using 3 static cycles of 7 minutes. The extracts were cleaned up on a multilayered column (30 cm × 1 cm i.d.), having Na₂SO₄, KOH silica, neutral silica, 44% acid silica, 22% acid silica, neutral silica and Na₂SO₄ from bottom to top. PCBs, OCPs and PBDEs were eluted with 80 ml of Hexane. The hexane extractable 'lipid content' of the samples was determined in a separate extraction in the ASE using the above conditions where the hexane was evaporated to dryness and the 'lipid content' was determined gravimetrically.

Sample analysis

All samples were analyzed for indicator PCBs, OCPs and PBDEs using a GCMS-QP2010 Plus operated in negative chemical ionization (NCI) mode. An Rxi® -XLB columns (fused silica) 0.28mm (i.d.)×25m fused silica capillary column was used. The injection port and transfer line temperatures were maintained at 270 °C and 280 °C, respectively, and the oven temperature program was 80 °C for 3 min, then 20 °C min⁻¹ to 320 °C and finally 320 °C for 6 min; total run-time 25 min. The mass spectrometer operating conditions were as follows: ion source 280 °C; ionization energy 38 eV; electron multiplier voltage set to 300 V. Selective Ion Monitoring (SIM) experiments were performed for each homolog.

Table 2 Concentration of indicator PCBs, PBDEs and OCPs in faeces from one infant (duplicate) and one toddler. And the ratios of them to the concentration in a pooled blood sample analyzed at 2008/9.

Compound		Concentration in Infant faeces * (ng/g lipid) CF _{i1} ±SD (CV)	Concentration in Toddler faeces (ng/g lipid) CF _{i2}	Concentration in Pooled Infants Blood [#] (ng/g lipid) CB _p	Infant faeces* to Pooled Blood [#] ratio CF _{i1} /CB _p	Toddler faeces to Pooled Blood [#] ratio CF _{i2} /CB _p
PCB	28	0.30 ± 0.06 (20)	0.59	1.2	0.25	0.49
	52	0.17 ± 0.01 (5.9)	0.35	n.d.(0.5)	-	-
	101	0.25 ± 0.02 (8.0)	0.30	n.d.(0.5)	-	-
	118	0.78 ± 0.06 (7.7)	1.8	1.4	0.55	1.3
	153	1.7 ± 0.10 (5.9)	1.7	3.4	0.51	0.49
	138	1.3 ± 0.23 (18)	2.2	2	0.65	1.1
	180	0.83 ± 0.01 (1.2)	0.60	2.3	0.36	0.26
PBDE	28	0.94 ± 0.93 (99)	0.19	0.5	1.9	0.38
	47	4.4 ± 0.31 (7.0)	5.0	13	0.33	0.38
	49	0.15 ± 0.01 (6.7)	0.15	n.d.(0.5)	-	-
	66	0.04 ± 0.01 (25)	0.04	n.d.(0.5)	-	-
	85	0.44 ± 0.40 (91)	0.62	0.50	0.88	1.2
	99	2.3 ± 1.3 (57)	3.9	4.8	0.90	0.81
	100	1.9 ± 0.35 (18)	3.4	3.4	0.54	1.00
	153	0.45 ± 0.23 (51)	1.0	3.2	0.14	0.31
	154	0.11 ± 0.08 (73)	0.32	0.50	0.21	0.64
	183	0.32 ± 0.39 (122)	0.04	0.50	0.63	0.08
DDT		0.70 ± 0.11 (16)	0.89	12	0.06	0.07
DDE		2.2 ± 0.24 (11)	3.1	280	0.01	0.01
HCB		0.52 ± 0.10 (19)	0.58	5.4	0.10	0.11
(γ-HCH) Lindan		0.40 ± 0.17 (43)	0.20	n.d. (2.4)	-	-
Heptachlor		0.70 ± 0.02 (2.9)	0.79	n.a.	-	-
t-chlordane		0.19 ± 0.08 (42)	0.37	n.a.	-	-
c-chlordane		0.24 ± 0.17 (71)	0.19	n.a.	-	-

*: Based on 2 replicate of a split sample

#: Concentration of p (ng/g lipid) for age group of 0-4 years in Australia for 2008/09 (Source: Chemical Monitoring Initiative: Australian human blood sample collection and chemical testing—Final Report

n.d. : not detected followed by the value in brackets which is the limit of detection;

n.a.: not analysed

Results and Discussion

7 PCBs, 10 PBDEs as well as a series of OCPs could be detected in the two faeces samples analyzed to date (Table 2). The mean CV for analyzing replicates of a pool of multiple samples collected from the infant was 34 % for the 24 compounds detected, where 15 of the 24 compounds had a CV < 28 % (ie. replicates deviate by less than a factor 1.5). Consistently good reproducibility was found for the PCBs whereas reproducibility was lowest for a range of the BDEs as well as chlordanes and HCH.

Overall the concentrations of POPs in the faeces from these one infant and one toddler were relatively similar and dominated by few PCBs (ie PCB 153, 138 and 118), BDE 47, 99, 100 and DDE which were found in the range of 1 to 5 pg/g lipid. These profiles in faeces are similar to that observed in pooled blood of children from Australia. In detail, on a lipid weight basis, concentration in the faeces of the main PCBs and BDEs in the infant range from about one third (BDE 47) to nearly equal (BDE 99) when compared to pooled blood collected in 2009 from 0 – 4 year old children. In contrast concentrations of OCPs were found at much lower levels than in the pooled blood (i.e. 1 – 10% for DDTs and HCB respectively).

The data also suggest that the levels of selected POPs were slightly higher in the faeces of the toddler compared with those from the infant which was somewhat unexpected. More work is required to assess whether this is a significant difference and if so, what factors this relates to (ie length of breast feeding, difference in intake)

On the whole our study showed that POPs can be identified using faecal samples collected from small children. The future goal is to assess whether we can use the concentration of POPs in faeces for estimating the concentration in blood lipid and therefore the body burden of POPs in the children. More work is underway to calibrate the underlying model where ideally we plan to obtain matched faeces and blood samples from same children..

Acknowledgements

The authors would like to thank the parents who participated in this research, and colleagues at EnTox and Queensland Health. Entox is co-funded by the Department of Health, Queensland.

References

1. Connell DW, Miller GJ, Anderson SM. (2007); *Developments in Environmental Science*, 7: 753-770
2. Siyali D. (1972); *Med. J. Aust* 2: 1063
3. Stacey CI, Thomas, BW (1975); *Pest. Monitor. J.* 9(2): 64
4. Matthew PL, Walter JR, George L. (1997); *Annual Review of Public Health*, 18: 211-244
5. Creel L. (2002); *Children's Environmental Health: Risks and Remedies. Population Reference Bureau, USA*
6. Clark NA, Demers PA, Karr CJ, Koehoorn M, Lencar C, Tamburic L. (2010); *Environ Health Perspect*, 118: 284-290
7. Dales R., et al.(2004); *Pediatrics*. 113: 628-631
8. Gavidia T, Pronczuk de Garbino J, Sly P. (2009); *BMC Pediatrics*. 9(1): 10
9. WHO Training Module. Children are not little adults. WHO training Package for the Health Sector, World Health Organization, www. Who. Int/ceh
10. Toms LML, Harden F, Paepke O, Hobson P, Mueller JF. (2008); *Environ. Sci, Technol.* 42: 7510-7515
11. Kissel JC, Robarge GM. (1988); *Chemosphere*.17: 2017-2027
12. Rohde S, Moser GA, Päpke O, McLachlan, MS.(1999). *Chemosphere* 38: 3397-3410
13. Abraham K, Knoll A, Ende M, Papke O, Helge H. (1996). *Pediatr. Res.* 40: 671-679
14. Moser GA, McLachlan MS.(2001); *Chemosphere*, 45: 201-211
15. Toms LML, Guerra P, Eljarrat E, Barcelo D, Harden FA, Hobson P, Sjodin A, Ryan E, Mueller JF.(2012); *Chemosphere*, 89: 398-403
16. Rohde S, Moser GA, Papke O, McLachlan MS. (1999); *Chemosphere*. 38(14): 3397-3410